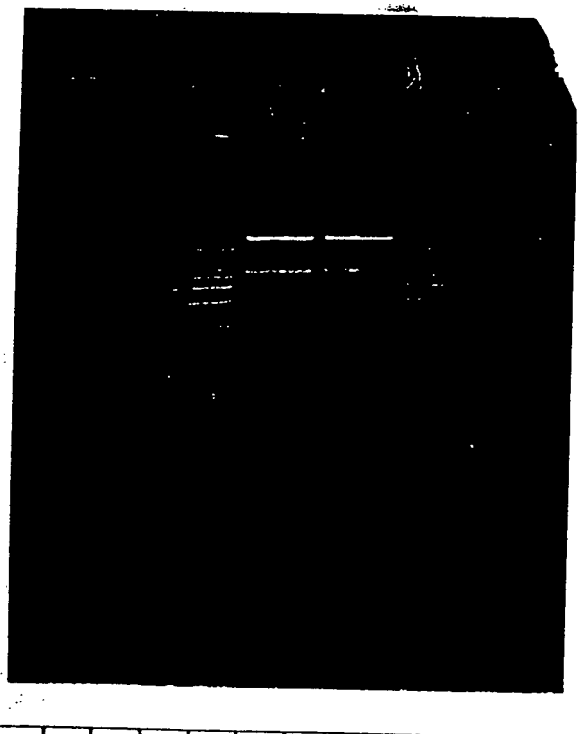
EcoRI / Bam HI RD's on λ Hep 5+6

en rxn mixed

10μl λ DNA
5μl 10x "B"
1μl EcoRI
1μl Bam HI
33μl H_2O
50

Inc 37°C ~2hrs → added 10μl 5x dye to each
2an 10μl each on 0.7% agarose (1x TBE)



Set up a 1% cmp gel for preparative electrophoresis on 2/20. Apparently λ 5+6 at least have the full length coding regions for Tyro 10.

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From Page No. 10

Ran remaining 50µl 1 5 & 6 RD's on 1% comparoose

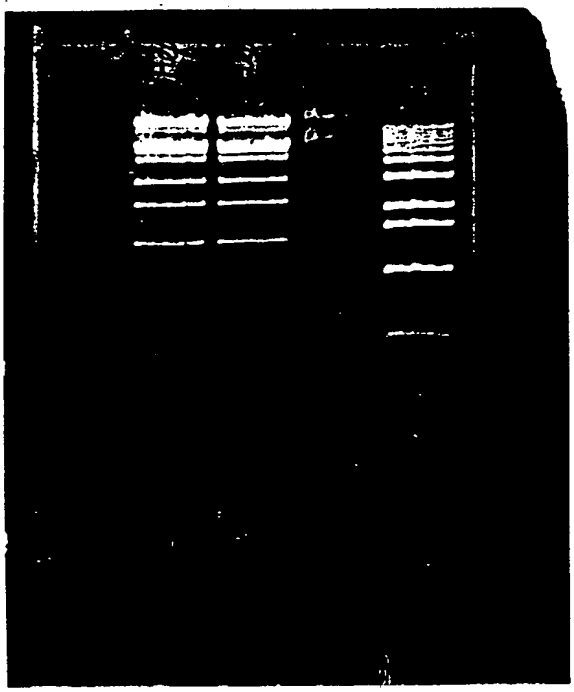
cut out indicated bands

Ran Magic PCR preps collected each in .5ml

combined.

Ligation to pRK5

mixed for each



1µl pRK5 ÷ EcoRI/BamHI
1µl 10x ligase buffer
1µl Ligase
7µl insert (or 1µl
6µl H_2O (or 6µl

Plus a vector alone ligation

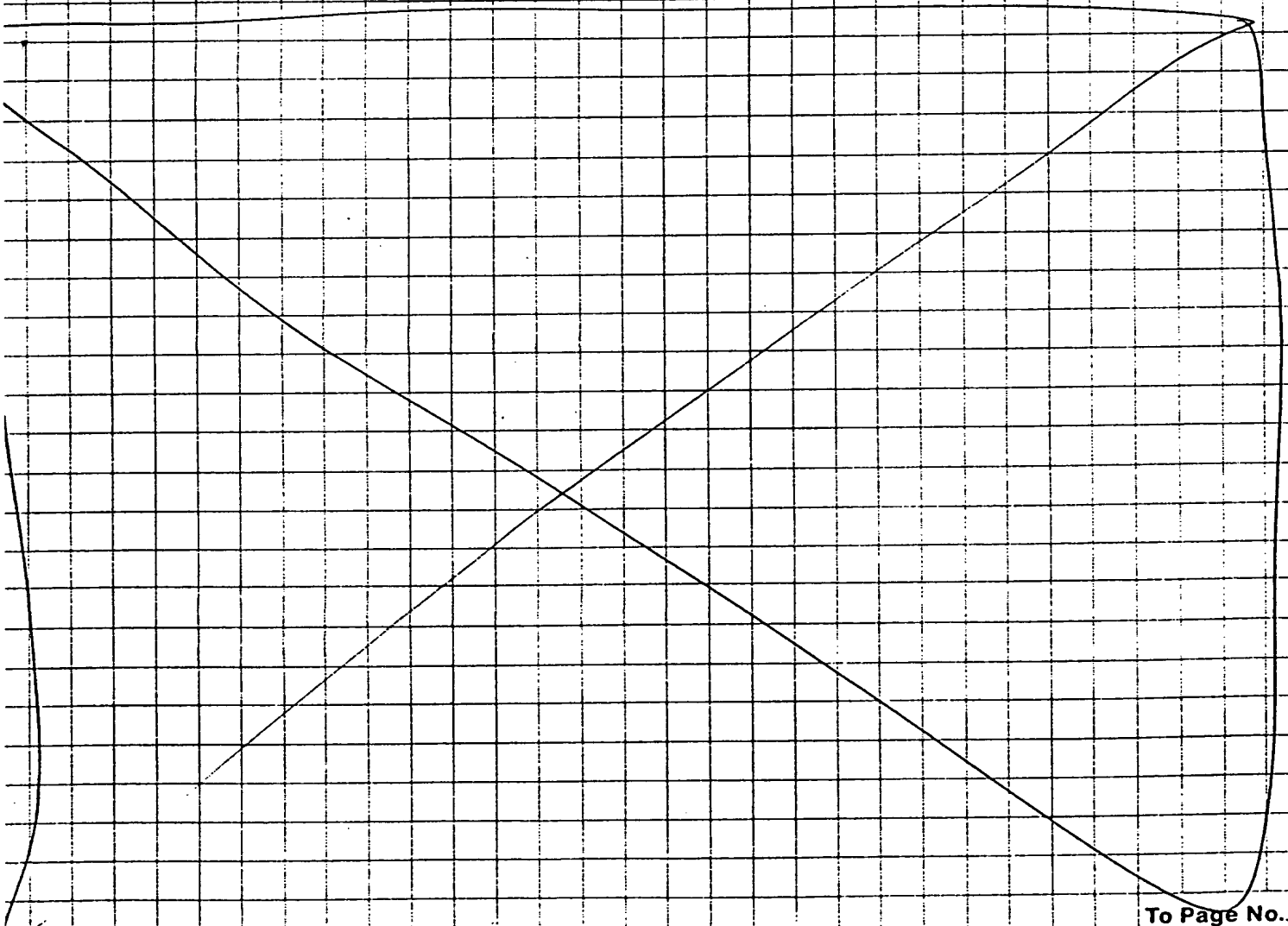
= 3 total.

Inc 12.5°C O/N.

To Page

Witnessed & Understood by me,	Date	Invented by J. Will Baron	Date [redacted]
		Recorded by [redacted]	

ransformed O/N pRES Liga to competent
XL-1 blue cells.
lated each onto 5 x 100mm LB carb 50 plates
(cont. plated onto 1 plate)
TNC all (11) 37°C O/N.



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Recorded by [redacted]

From Page No. 12

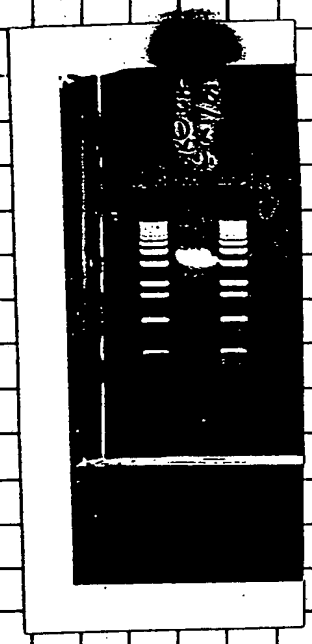
RD of SKG/CH2CH3 IgG1 fusion vector

Mixed

2µl DNA (~4µg)
5µl 10x "B"
4µl H_2O
1µl EcoR1
1µl BstEII
50

Inc 37°C ~ 2hrs

Ran on 0.7% agarose



Isolated band on NA45 paper
Eluted in 400µl 1M NaCl (in TE) 70°C 2
Removed paper.
Stored -70°C O/N.

Ordered PCR primers to PCR ECD of HPI
& Incorporate a BstEII site @ 3'e

Genentech, Inc.
Genentech, Inc.
Genentech, Inc.

SYNTHETIC DNA REQUEST A-6749 ✓

REQUESTOR	EXTENSION	LAB NO.	PROJECT NAME OR NUMBER	DATE NEEDED	AMT NEEDED
WILL BARON	2650	10231	1713		

LIST SEQUENCE(S) 5' to 3' & NOTE AS SUCH
SIZE & FRAGMENT NAME:
1x 29mer
1x 35mer

PLEASE INDICATE BY **** FRAGMENT(S) TO BE CLONED

① Tyro P13 (29mer)
5' TGG·GAG·GGG·GAG·CCC·ATG·CGC·CAC·3' [redacted]

② Tyro P14 (35mer)
5' GTA·CGG·TNA·CCG·GCG·GTC·GGG·CTC·
·CCC·TCG·GGC·TT·3' [redacted]

FRAGMENT USE: ☐ PRIMER ☐ PROBE ☒ PCR ☐ GENE CONSTRUCTION ☐ LINKER/ADAPTOR
☐ MUTAGENESIS ☐ OTHER (SPECIFY):

SPECIAL REQUESTS

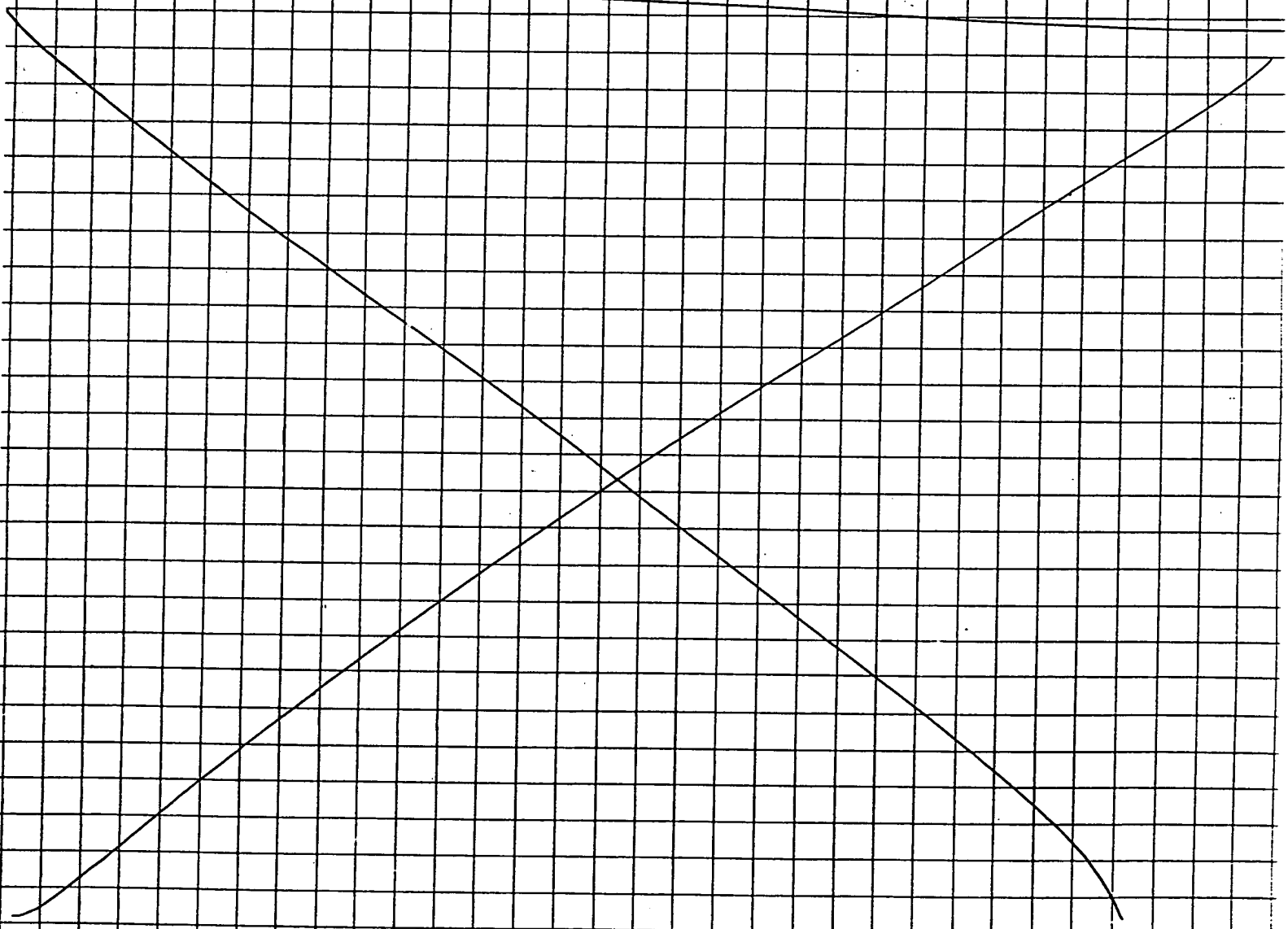
REQUESTOR Will Baron DATE [redacted] APPROVED Parker DATE [redacted]

To Page N

Witnessed & Understood by me, | Date | Invented by Will Baron | Date [redacted]
Recorded by [redacted]

om Page No. 13

Checked pRK5/HPTK6 (tyro10) transformation plates
Got 39 transformants
Started 39 x 5ml LB cam 50 (+ master plate)
Inc all 37°C O/N.



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From Page No. 14

Did Magic MP's on 12 of O/N pRK5/HPTK6 MP's (Stored remaining 27 cultures + master @ 4

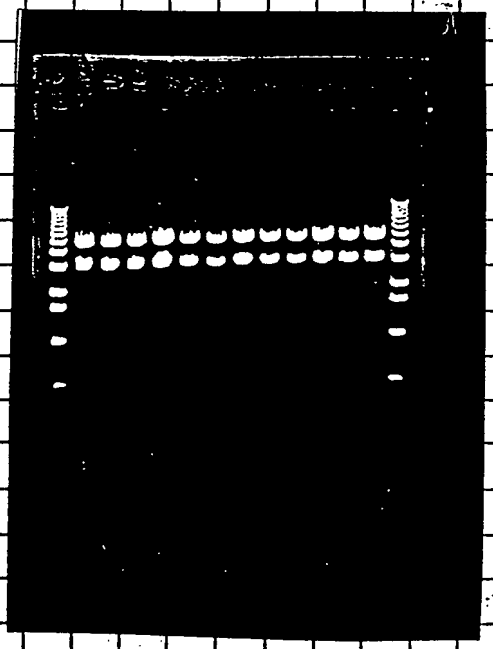
Collected each in 50µl TE

RD's

Per rxn
2µl MP DNA
2µl 10x "B"
15µl H_2O
0.5µl EcoRI
0.5µl BamHI
20

Inc 37°C ~ 1.5 hrs → added 4µl dye to each

Ran ~ 15µl each on 0.7% agarose (1x TBE)



All positives.

Started 1x 500ml 2YT each 50 Midi prep on #1

Inc 37°C O/N.

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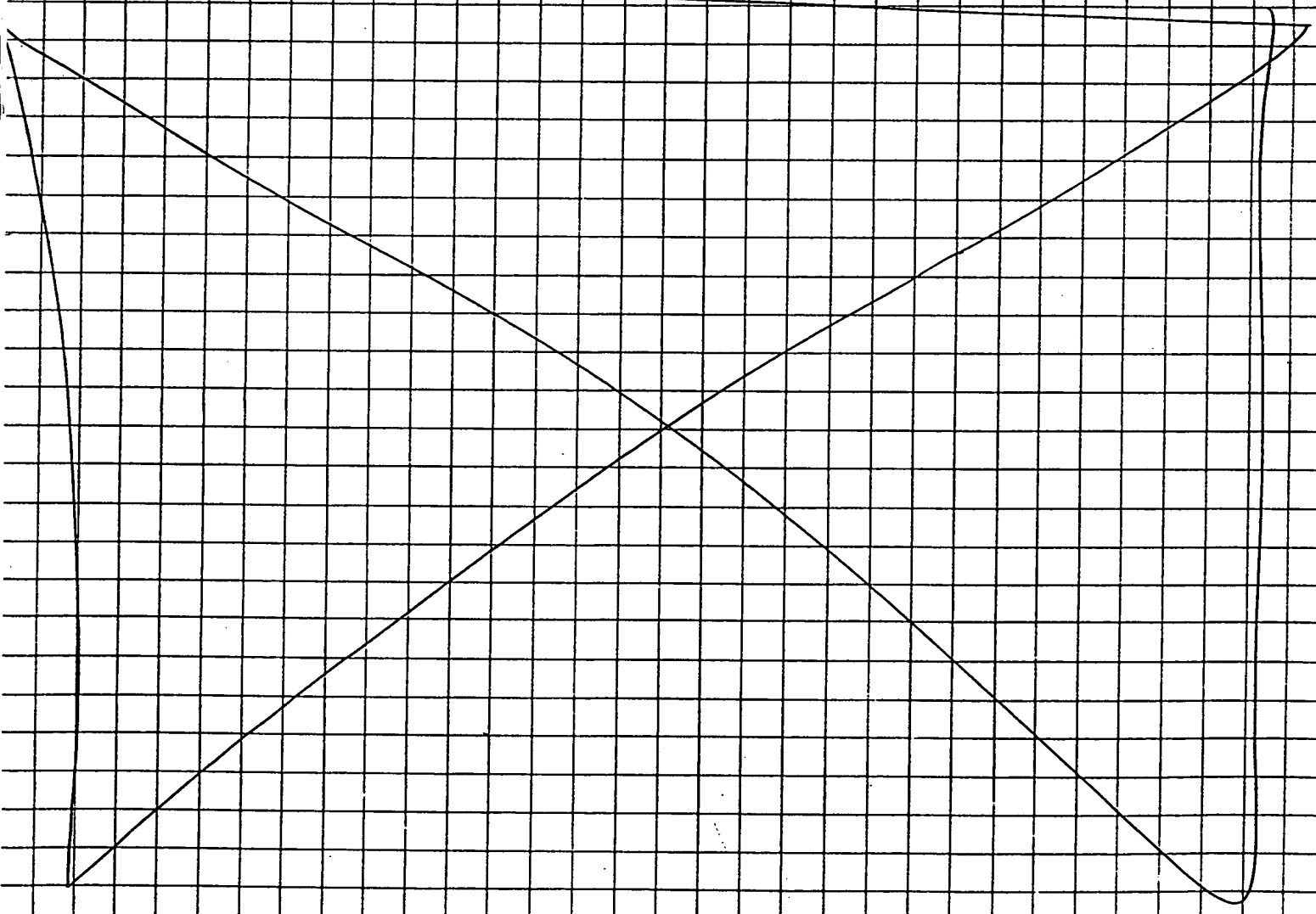
m Page No. 15

Stored 1.2ml O/N HPTK6/pRK5 + 400µl 50% glycerol @ -70°C

Did Magic Maxi prep on remainder of culture. EtOH ppt'd DNA after elution from resin column. Stored ppt'd pellet in -20°C 70% EtOH. O/N



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nessed & Understood by me,	Date	Invented by EWM Eaton	Dat [redacted]
		Recorded by	